## REMARKS

Claims 2, 3 and 5-35 are active in this application.

Applicants wish to thank Examiners Qian and Yucel for the interview granted to the Applicants' undersigned representative on October 7, 2002.

During this discussion, the undersigned argued that based on the success of the change in codons in the jellyfish green fluorescent protein (as described in Zhang), which is more evolutionary related to mammals than bacteria, there would be no expectation that making similar changes in the bacterial protein would have any positive effect as demonstrated in the data provided in the specification.

Applicants further note that the present claims, for example, as claimed in Claim 2, are directed to a polynucleotide comprising a modified Cre recombinase gene modified such that it is expressed in elevated levels compared to the unmodified Cre recombinase gene and an inducible promoter operatively linked to the modified Cre recombinase gene. The advantage of the claimed invention is a high expression efficiency of the modified Cre recombinase when coupled to the inducible promoter.

In Example 2 of the specification, a modified (mammalian) Cre gene or a wild type Cre gene was inserted into a vector with a constitutive promoter (CAG promoter-pCXN vector) or an indicible promoter, which relative the constitutive promoter is weak (thymidine kinase promoter-pMC1 vector). These vectors were transfected into CHO cells to express the recombinase and used to assess the frequency of recombination and expression using the green fluorescent protein expressed from the pCXN-YK1 vector. The results of these experiments are presented in Figure 6 of this application.

Comparison of the strong and constitutive promoter (pCXN Figures 6A and B) and the inducible promoter (pMC1 Figures 6C and D) shows that the modified Cre gene exhibits

a higher recombination frequency relative to the wild type Cre gene, which is indicative that the modified type Cre gene has a higher level of expression relative to the wild type gene.

Focusing on the wild type Cre gene (□, ○, Δ) using the inducible promoter vector (Figures 6C and D), the recombination efficiency was significantly lower compared to the constitutive promoter vector (Figures 6A and B). This result can be attributed to the lower expression efficiency using the inducible promoter relative to the constitutive promoter.

However, focusing on the modified Cre gene (■, ●, ▲) using the inducible promoter vector (Figures 6C and D), the recombination efficiency was substantially the same relative to constitutive promoter (Figures 6A and B). In other words, the inducible promoter had a significant effect on the recombination efficiency observed using the modified Cre gene relative to the wild type gene and certainly could not have been predicted from the data using the wild type Cre gene, where there were significant differences in the recombination efficiency relative to the promoter included in the vector.

Therefore, the combination of the inducible promoter with the modified Cre gene provides the advantage of specifically regulating the expression of the gene in, for example, a time and/or location-specific manner, while not compromising the expression of the gene and the recombination efficiency obtained with the Cre recombinase. Furthermore, the references cited in the pending Office Action do not describe the specific combination of a modified Cre recombinase with an inducible promoter as claimed nor do the cited references provide any expectation for the advantages obtained by this combination. In addition, Applicants note that Claims 25-35, which depend from Claim 2, define the modified Cre recombinase gene as comprising SEQ DI NO:1. SEQ ID NO:1 is neither described or suggested by the cited references.

In view of the above, Applicants request reconsideration and withdrawal of the rejection of Claims 1-4 and 7 under 35 U.S.C. § 103(a) over St-Onge et al and Bergemann et al in view of Zhang and Nakamura et al.

Also, during the discussion with the Examiners, the Applicants' undersigned representative pointed out support for the phrases "location-specific" and "time-specific" on page 9 of the specification. In addition, Applicants attach hereto published scientific articles demonstrating the common usage of these phrases. Mohan et al (Biology of Reproduction 67, 447-453 (2002)) in the Abstract in sentence one indicates the use of time and location-specific expression of appropriate genes. In addition, Fahlen et al (EUR. J. Immunol. 1997. 27:2057-2065) describes the use of location-specific regulation of specific genes. The remaining issues under 35 U.S.C. § 112, second paragraph are believed to have been addressed by amendment.

Therefore, withdrawal of the rejection of Claims 5-11 under 35 U.S.C. § 112, second paragraph is requested.

The issue of enablement under 35 U.S.C. § 112, first paragraph relative to Claim 8 was also discussed. During this discussion, the Examiner indicated that a claim including the phrase "wherein an active Cre recombinase is expressed" would be favorably reconsidered. Claim 8, as well as Claims 9-11, include this phrase. Therefore, favorable reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph is requested in light of the amendment submitted herein.

The rejection of Claim 8 under 35 U.S.C. § 101 is believed to have been obviated by amendment.

Applicants submit that the present application is now ready for allowance. Early notification of such allowance is kindly requested.

Respectfully submitted,

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IN THE CLAIMS

--1. (Cancelled)

2. (Amended) A polynucleotide comprising [the] a modified Cre recombinase gene [for mammals according to claim 1] which is modified such that it is expressed in elevated levels compared to the unmodified Cre recombinase gene; and an inducible promoter operatively linked to the modified Cre recombinase gene.

3. (Amended) The polynucleotide according to claim 2, further comprising at least one of [following sequences;

(1) regulatory sequences operatively liked to the modified Cre recombinase gene for mammals,

(2)] a marker gene,

[(3) ]a nucleic acid encoding a nuclear transport signal, and

[(4)] a Kozak sequence.

4. (Cancelled)

5. (Amended) The polynucleotide according to claim [4] 2, wherein the inducible promoter is a location-specific promoter.

6. (Amended) The polynucleotide according to claim [4] 2, wherein the inducible promoter is a time-specific promoter.

7. (Twice Amended) A polynucleotide complementary to the polynucleotide according to Claim [1] 2.

8. (Twice Amended) [An] A non-human animal into which the gene encoding the polynucleotide according to Claim [1] 2 is introduced, wherein an active Cre recombinase is expressed in the animal.

9. (Twice Amended) An organ into which the gene encoding the polynucleotide according to Claim [1] 2 is introduced, wherein an active Cre recombinase is expressed in the organ.

10. (Twice Amended) A tissue into which the gene encoding the polynucleotide according to Claim [1] 2 is introduced, wherein an active Cre recombinase is expressed in the tissue.

11. (Twice Amended) A cell into which the gene encoding the polynucleotide according to Claim [1] 2 is introduced, wherein an active Cre recombinase is expressed in the cell.

12. (Twice Amended) A method of knocking-in a desired gene in a location controlled and/or time-controlled manner; comprising the steps of:

(1) introducing a first gene construct and a second construct into cells, tissues, organs or whole bodies,

wherein the first gene comprises a polynucleotide according to Claim [1] 2 [and an inducible promoter for inducing expression of the polynucleotide at a site into which the desired gene is to be knocked-in, in a location-controlled and/or time-controlled manner]; and the second gene construct comprises a first loxP sequence, a second loxP sequence located downstream of the first loxP sequence, a second promoter sequence located upstream of the first loxP sequence, and the desired gene located downstream of the second loxP sequence,

(2) expressing a Cre recombinase gene by the inducible promoter in a location-controlled and/or time-controlled manner, and

(3) placing the desired gene under control of the promoter sequence in the second gene construct by [means of] site specific recombination on the second gene construct by Cre recombinase expressed in step (2), thereby knocking-in the desired gene in a location-controlled manner and/or time-controlled manner.

13. (Twice Amended) A method of knocking-out a desired gene in a location controlled and/or time- specific manner; comprising the steps of:

(1) introducing a first gene construct and a second gene construct into cells tissues organs or whole bodies,

wherein the first gene construct comprises a polynucleotide according to Claim 2[1 and an inducible promoter for inducing expression of polynucleotide at a site into which the desired gene is to be knocked-out, in a location-controlled and/or time-controlled manner]; and the second gene construct comprises a first loxP sequence, a second loxP sequence located downstream of the first loxP sequence, a promoter sequence located upstream or downstream of the first loxP sequence, and the desired gene located downstream of the promoter and the first loxP sequence,

(2) expressing a Cre recombinase gene by the inducible promoter in a location-controlled manner, and

(3) [falling off] inserting a part or whole of the desired gene from the second gene construct by [means of] site specific recombination [on] with the second gene construct mediated by Cre recombinase expressed in step (2), thereby knocking-out at least a part or whole of the desired gene, in a location-controlled and/or time-controlled manner.

14. (Amended) The method of claim 12, wherein the desired gene is selected from the group consisting of a xenograft antigen, carcinogenic antigen, and anti antibody-production-associated-molecule antibody.

18. (Amended) An organ [taken out] from the transgenic animal according to claim 16.

19. (Amended) A tissue [taken out] from the transgenic animal according to claim 16.

20. (Amended) A cell [taken out] from the transgenic animal according to claim 16.

21. (Amended) A method for treating a disease caused by malfunction of an organ, comprising a step of transplanting the organ according to Claim 18, into an organism.

22. The method according to Claim 13, wherein the desired gene is selected from the group consisting of a xenograft antigen, carcinogenic antigen, and anti antibody-production-associated-molecule antibody.

23. (Amended) A method for treating a [desease] <u>disease</u> caused by malfunction of a tissue, comprising a step of transplanting the tissue according to Claim 19 into an organism.

24. (Amended) A method for treating a [desease] <u>disease</u> caused by malfunction of a cell, comprising a step of transplanting the cell according to Claim 20 into an organism.

25.-35. (New)--